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Costs of pair-bonding and paternal care in male prairie voles (*Microtus ochrogaster*)

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ABSTRACT

The direct costs of paternal care are relatively well documented in primates, however little research has explored these effects in monogamous rodents. The present study examines the long-term effects that pairing and parenting have on male prairie voles. We hypothesized that there would be a significant weight loss over the course of pairing and parenting, presumably from the energetic demands that accompany these changes in social condition. In a longitudinal study, we followed ten male prairie voles through being housed with their brother; paired with a female; and caring for three consecutive litters. We found a significant drop in bodyweight across time, with maximum weight loss near the weaning of the first litter. At that same time, feeding increased, leading to possible recovery in weight; however, leptin levels dropped precipitously across time and did not recover. Corticosterone did not change significantly across time points, and overall activity levels also did not vary significantly over the course of the study. In addition, newly paired males showed a significant increase in preference for a 2% sucrose solution during a three-hour test, indicating a metabolic need for more calories. A cross-sectional study confirmed leptin and corticosterone findings, and showed significant loss of subcutaneous (inguinal) fat in males that had cared for a litter of pups, when compared to males housed with their brothers or newly paired males. These results suggest that cohabitation with a female, and caring for pups, all have costs for male prairie voles.

1. Introduction

Monogamous mammals display a suite of behaviors, including pair-bonding, intrasexual aggression, and parental care by fathers [1–3]. Paternal care in monogamous rodents includes direct behaviors, such as caring for the offspring through huddling, retrieving to the nest and grooming, and indirect behaviors, such as ensuring the continued investment of the female in her litter. Consequently, paternal care in monogamous rodents is often crucial, or at least beneficial, for survival of the offspring [4,5]. In prairie voles (*Microtus ochrogaster*), fathers display a suite of parental behaviors very similar to mothers [6]. In addition, females may lose pregnancies if their mates are not present [7], pups with both parents are left alone in the nest less often [8], and pups in groups with more adults have higher survivorship [9]. In studies of prairie voles where direct benefits of father presence were *not* found, authors have suggested that environmental conditions were not particularly challenging [8,10]. However, there are still presumably energetic costs associated with the parental care given by fathers, just as there are for mothers [11,12]. It is intriguing that wild, adult male prairie voles have higher survivorship when living singly than when living in male–female pairs, which suggests a possible survivorship consequence of pairing and parenting [10].

Food availability is clearly one of the primary factors limiting reproduction in both males and females. Gestation, lactation and infant care are all energetically expensive [11], and males as well as females have shown reduced ability to reproduce when under food restriction [13,14]. The current "metabolic fuels" hypothesis [15,16], which is based on rat studies, suggests that effects of food availability on reproductive readiness and feeding behavior are more immediately reliant on glucose levels, rather than stored fat reserves. However, monogamous male rodents are in a fairly unusual situation for a male mammal. Once mated, female prairie voles may give birth every three weeks, given a successful postpartum estrus. Therefore, male prairie voles are constantly caring for infants and may more heavily rely on longer term energy reserves and a regulatory system that integrates long-term energy signals (e.g., leptin) in order to meet the demands of this important behavior.

Although there is little research on the costs associated with paternal care in monogamous rodents, this topic has been addressed in monogamous primates. Schradin and Anzenberger [17] found that both male and female common marmosets (*Callithrix jacchus*) had lower leaping ability while carrying infants, although the carriers did not lose weight during the period of infant care (thus the lessened leaping ability was presumably due to the extra weight of the infants). Both common marmoset and cotton-top tamarin (*Saguinus oedipus*)

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males gained significant amounts of weight prior to the birth of their infants, presumably to prepare for the energetic demands of carrying [18]. Male cotton-top tamarins lost up to 11.3% of their pre-birth weight during the carrying of their offspring or non-littermate infant siblings [19]. In cotton-top tamarins, there was also a correlation between weight lost and time spent carrying the infant, with those animals that provide the most care losing the most weight [20]. In male fat-tailed dwarf lemurs (*Cheirogaleus medius*), which are also socially monogamous, fathers tended to have less stored fat in their tails than non-fathers during the period of infant care [21].

Male canids also perform many paternal care behaviors which would appear to be energetically expensive (ex. regurgitation of food, provisioning of solid food to young), and presumably beneficial to young [22,23]; however there are also documented cases of single canids raising young successfully [24].

Although the energetic demands associated with investing in parental care might tax energy reserves, decreases in energy reserve might also result from reductions in energy intake. Infant care in primates has been shown to decrease time spent foraging for food, foraging efficiency (food gained per unit time), time locomoting, and time spent in social activities [25,26]. This effect on foraging is even more prominent in animal species that rely on crypticity to avoid predation versus animals with other anti-predation strategies [27]. In this study we sought to explore costs associated with long-term energy balance of male prairie voles of engaging in a pair bond and participating in infant care. Furthermore, we tested the hypothesis that changes in these behaviors were related to two metabolically important hormones, corticosterone and leptin.

Our overarching hypothesis was that paternal males, like mothers, would experience an energetic cost to raising infants and that this cost would be associated with indices of long-term energy balance. We predicted that throughout the process of pair-bonding and parenting, males would demonstrate higher metabolic needs ("costs") than unpaired males. We hypothesized that as males underwent these changes in social behavior, body weight and fat reserves would drop and activity levels would increase. Plasma leptin might be expected to drop along with body weight; we included its analysis to give us information about whether or not weight loss was due to loss in fat reserves. Since sucrose is calorically dense and highly lipogenic, we also expected sucrose intake to increase during pairing and parenting as a possible response to increased metabolic need. The alternative hypothesis was that males, unlike females, do not experience sufficient costs of social behavior that reflect changes in any of these behavioral or energetic measures. We first addressed these questions in a longitudinal study in which we followed males through different social conditions. We followed up with a second, cross-sectional experiment, in which we examined whether or not losses in body weight and leptin levels observed in the longitudinal experiment might be due to losses in body fat. To our knowledge, this is the first time that these questions have been addressed in monogamous rodents.

2. Methods

2.1. Experiment I: Longitudinal study of effects of male pairing and parenting

2.1.1. Subjects

Subjects were ten laboratory-bred adult male prairie voles (*M. ochrogaster*), descended from a colony originally captured in Illinois. Subjects were housed with parents in large polycarbonate cages (44 cm long × 22 cm wide × 16 cm high) for the first 21 days after birth, and then housed with same sex siblings in standard mouse cages (27 cm long × 16 cm wide × 16 cm high) until being used for this study. All subjects were maintained on a 14 hour light:10 hour dark cycle, with lights on at 0600 and lights off at 2000 h. Voles were housed in a colony room with an average temperature of 68–72°. Cotton was provided as nesting material, and food (Purina high-fiber rabbit chow) and water were available *ad libitum* throughout the study. All procedures were approved and annually reviewed by the Institutional Animal Care and Use Committee of the University of California, Davis.

2.1.2. Treatments

Male prairie voles were removed from small polycarbonate cages and placed in a large breeder cage one month before the first data collection point to control for activity levels and weight fluctuations due to available space. During this time, they were housed with another male (either a sibling or unrelated male that they had been housed with since weaning). During this time, they were exposed to 2% sucrose dissolved in distilled water for 48 h in order to acclimate them to sucrose. For all ten males, data were collected at five time points: males housed with sibling, three days after being paired with a female, 17 days post-pairing and therefore before the birth of the first litter, 17 days after the birth of the first litter and therefore before the birth of the second litter, and 17 days after the birth of the second litter and therefore shortly before the birth of the third litter. The 17 day time point was chosen to represent a time point in which the majority of male investment had already gone into a litter, but which was not so close to the birth as to be disruptive (average days to first litter for voles in this colony, 29.9 ± 2.5 days; average days between litters, 24.1 ± 1.3 days; Stone and Bales, unpublished data). At each time point, there were three days of testing during which body weights were recorded, blood samples were taken, 24-hour activity levels were recorded, and males received a three-hour sucrose preference test.

2.1.3. Data collection

On the first day of testing for each time point, behavior was recorded for 24 h on time-lapse video. All members of the cage were placed in a clean cage with new bedding. The male was collared for identification on the video, and placed in the cage before the rest of the members of the cage. The video was later scored for motor behavior, feeding, and drinking behavior using Behavior Tracker 1.5 (www.behaviortracker.com). The tape was run for 24 h straight at a compression of 0.2 to 1. Activity levels were quantified by separating the cage into 4 sections, and quantifying the number of times the subject's front paws crossed into a new section. Durations of feeding and drinking behavior were also recorded. For the first 3 h of the tape, we also quantified social behaviors including nest-building, grooming, and huddling with the mate and/or offspring. Red lights were placed behind the cage so that during the dark period of the day behaviors may still be recorded on the video. Low intensity red light was used in order to avoid interfering with normal nighttime behavior and circadian rhythm as circadian responses to light appear to be highest in the middle of the color spectrum (Provencio and Foster, 1995). At the end of the 24 hour period the collar was removed, the males were weighed, placed back in the cage and the cage was placed back in the animal room until the next test.

On the second day of testing for each time point, each male received a sucrose preference test. A clear separator, with small holes to allow touch and social interaction, was placed between the male and other occupants in the cage (ex. female and offspring when present). However, only the male had access to the sucrose and water bottles on his side of the cage. The male was given two bottles, one containing 200 ml of distilled water, and the other containing 200 ml of 2% sucrose by weight in water. The subject was allowed to consume as much of either liquid as desired for 3 h, at which point the bottles and the cage divider were removed. The amount of each liquid consumed was recorded.

On the third day of testing at each time point, a blood sample (300 µl) was taken from each male via a supraorbital bleed. The vole

was placed under anesthesia using gaseous isoflurane initially at 10% and maintained during the bleed at 2.5–3.5%. All bleeds were performed between 1000 and 1200 h to control for effects of time of day on serum corticosterone levels. The blood was centrifuged at 3200 rpm at 0–4 °C for 5 min and the plasma frozen at −80 °C until processing for leptin and corticosterone.

2.1.4. Corticosterone assay

Corticosterone was assayed using a commercially available radioimmunoassay from MP Biomedicals in Irvine, Ca. This assay has been validated for use on prairie voles in a previous study [28]. Samples were assayed after a 1:2000 dilution in steroid buffer. Intra-assay CVs averaged 2.75%. There is no inter-assay CV as all samples were assayed at once.

2.1.5. Leptin assay

Leptin was assayed using a commercially available multiple species assay kit (Millipore Inc., Billerica, MA), chemically validated for prairie voles in our laboratory by assessing parallelism and quantitative recovery. A 1:1 or 1:2 dilution would have been preferable due to the low levels of leptin in some samples. However, due to small sample volume necessary for survival eye bleeds, it was necessary to assay at a 1:4 dilution. Values for samples in which leptin levels were below assay detection were set to the low limit of the assay (0.5 ng/ml). Intra-assay CVs averaged 3.28%. There is no inter-assay CV as all samples were assayed at once.

2.1.6. Data analysis

Data analysis for all variables was carried out by analysis of variance (ANOVA) using SAS 8.2, with male identity as a random variable to account for repeated measures [29]. Following a significant ANOVA, post hoc tests were carried out using a least-squared means test. All data were examined for assumptions of ANOVA including normality and homogeneity of variance. All tests were two-tailed and significance was set at $p<0.05$. Correlations between variables were examined using Pearson's correlations.

Power equations were taken from Diggle et al. [30], with standard deviation based on feeding behavior data, for five longitudinal observations. Using $\alpha=0.05$ in a two-tailed test, our equations also yielded an 80% power to detect a 2-fold change in feeding using eight subjects. Using ten subjects, a 1.7-fold change in behavior was detectable.

2.2. Experiment II: Cross-sectional study of effects of male pairing and parenting

2.2.1. Subjects

Subjects were 30 male prairie voles housed as described above.

2.2.2. Treatments

Males were divided into three groups: males housed with sibling, three days after being paired with a female, and 17 days after the birth of the first litter and therefore before the birth of the second litter. Two males in the third group failed to produce infants, thus resulting in a sample size of eight for that group.

2.2.3. Data collection

All males were euthanized between the ages of 104 and 120 days. At time of euthanasia, blood was collected and processed as described above. Voles were weighed and the following were dissected out and weighed: perirenal fat pad, subcutaneous fat pad, epididymal fat pad, and adrenal gland. Leptin assay was performed as described above with an intra-assay c.v. of 2.80%. Corticosterone assay was performed as described above with an intra-assay c.v. of 3.31%.

3. Results

3.1. Experiment I—Longitudinal study

3.1.1. Body weight

Social condition significantly affected body weight ($F_4=3.15$, $p=0.026$), with males weighing significantly less right before the birth of the second litter (Fig. 1). Post hoc testing revealed that there was significant differences between unpaired and males before the birth of both their second and third litters (from 2nd litter, $t=2.910$, $p=0.006$; from 3rd litter, $t=2.648$, $p=0.012$). There were also significant differences between newly paired males and males before the birth of their second litter ($t=2.293$, $p=0.028$). The difference between newly paired males and males before the birth of their third litter approached significance ($t=2.023$, $p=0.051$).

3.1.2. Corticosterone

Differences in corticosterone were not statistically significant ($F_4=0.89$, $p=0.482$). Corticosterone levels were: unpaired, 1351.51 ± 160.1 ng/ml; newly paired, 1313.98 ± 159.4 ng/ml; before birth of first litter, 1349.66 ± 103.5 ng/ml; before birth of second litter, 1365.27 ± 67.9 ng/ml; before birth of third litter, 1522.04 ± 107.5 ng/ml. There was no correlation between corticosterone levels and leptin levels ($r=0.066$, $p=0.665$). There was, however, a trend towards a negative correlation between corticosterone and bodyweight ($r=-0.261$, $p=0.073$).

3.1.3. Sucrose preference

Absolute measures of sucrose consumption were not significantly affected by social condition ($F_4=2.24$, $p=0.085$). However, preference of sucrose over water expressed as a ratio of sucrose consumed to total liquid consumed was significantly affected by social condition ($F_4=4.42$, $p=0.006$; Fig. 2). In post hoc tests, sucrose preference of unpaired males was significantly lower than all other time points (difference from newly paired, $t=3.13$, $p=0.003$; from before the birth of the first litter, $t=3.531$, $p=0.001$; from before the birth of the second litter, $t=2.29$, $p=0.029$; from before the birth of the third litter, $t=3.65$, $p<0.001$).

3.1.4. Activity/locomotor levels

The overall ANOVA was not significant for activity levels ($F_4=2.04$, $p=0.113$). However, overall activity was negatively correlated with bodyweight ($r=-0.334$, $p=0.022$).

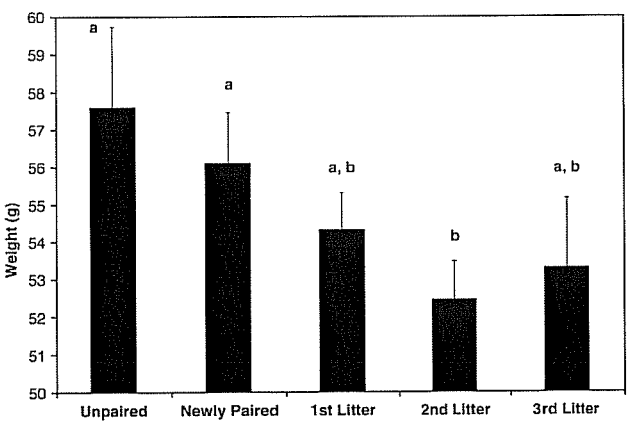

Fig. 1. Effects of social condition on weight of males (mean ± S.E.; $n=10$ for all groups). Groups that are statistically different according to post hoc testing are marked with different letters; groups marked with the same letter are not significantly different. Post hoc analysis for unpaired versus 3rd litter was nearly significant ($t=2.02$, $p=0.051$).

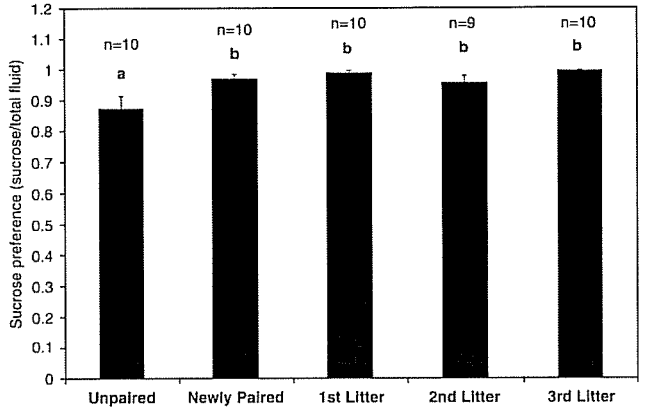

Fig. 2. Effects of social condition on sucrose preference as a ratio of total fluid intake (mean ± S.E.). Groups which are statistically different according to post hoc testing are marked with different letters: groups marked with the same letters are not statistically different.

3.1.5. Feeding behavior

Feeding behavior changed as a result of change in social condition ($F_4 = 5.79$, $p = 0.001$; Fig. 3). Post hoc analysis showed a difference between unpaired males and all other time points with the exception of males before the birth of their second litter (from newly paired, $t = 4.16$, $p = 0.0002$; from before the birth of the first litter, $t = 4.03$, $p < 0.001$, from before the birth of the third litter, $t = 2.62$, $p = 0.013$). However, differences between the unpaired males and males before the birth of their second litter did approach significance ($t = 1.96$, $p = 0.059$). There were also significant differences between newly paired males and males before the birth of the second litter ($t = 2.12$, $p = 0.042$). Males before the birth of their first litter and males before the birth of their second litter also differed significantly ($t = 2.10$, $p = 0.043$). Feeding was also positively correlated with bodyweight ($r = 0.444$, $p = 0.002$).

3.1.6. Drinking behavior

The duration of drinking behavior was not significant across social condition ($F_4 = 0.73$, $p = 0.579$). Drinking behavior was positively correlated with activity levels ($r = 0.297$, $p = 0.043$).

3.1.7. Social behavior

There were no significant changes in social behavior across social condition, although there were some suggestive trends. There was a trend for grooming to differ across social condition [$F_4 = 2.14$, $p < 0.098$], with the unpaired group displaying the highest levels of grooming (Table 1). Nest-building also tended to vary across social condition [$F_4 = 2.47$, $p < 0.063$]. In this case the least amount of time was spent in nest-building by the unpaired males. Time spent huddling with cage-mates did not differ by social condition [$F_4 = 1.10$, $p < 0.372$].

Table 1
Means (± standard errors) of social behaviors displayed in different conditions (minutes per 3 h of observation).

	Unpaired	Newly paired	First litter	Second litter	Third litter
Groom	5.5 ± 1.3	2.9 ± 1.1	2.6 ± 0.7	3.1 ± 0.9	1.7 ± 0.3
Huddle	66.0 ± 2.6	66.5 ± 4.4	66.3 ± 7.2	60.9 ± 7.3	80.0 ± 6.6
Build nest	20.1 ± 1.3	36.4 ± 5.5	24.6 ± 4.8	31.6 ± 6.4	28.4 ± 6.0

3.1.8. Leptin levels

The effects of social condition on leptin levels were significant [$F_4 = 21.31$, $p < 0.0001$; Fig. 4], with leptin dropping over time. Post hoc analysis showed a difference between unpaired males and all other time points (from newly paired, $t = 4.20$, $p = 0.0002$; from before the birth of the first litter, $t = 6.03$, $p < 0.0001$; from before the birth of the second litter, $t = 8.07$, $p < 0.0001$; from before the birth of the third litter, $t = 7.42$, $p < 0.0001$). There were also differences between newly paired males and both males before the birth of their second litter ($t = 4.133$, $p < 0.001$) as well as males before the birth of their third litter ($t = 3.64$, $p < 0.001$). Males before the birth of their first litter also differed significantly from males before the birth of their second litter ($t = 2.304$, $p = 0.028$). Finally, there was a significant negative correlation between absolute sucrose consumption and leptin ($r = -0.340$, $p = 0.020$), but not sucrose preference and leptin ($r = -0.208$, $p = 0.166$).

3.2. Experiment II—Cross-sectional study

3.2.1. Fat and adrenal weights

Fat pad weights are shown in Fig. 5. Social condition significantly affected subcutaneous fat pad weight ($F_2 = 3.84$, $p = 0.036$). Post hoc testing revealed a significant decrease in subcutaneous fat pads of paired males 17 days after the birth of the first litter when compared to unpaired males ($t = 2.733$, $p = 0.012$). Epididymal fat pad weight was also reduced in newly paired males, but this did not reach statistical significance ($F_2 = 3.02$, $p = 0.067$). The difference in adrenal gland weight (Fig. 6) between unpaired and newly paired males

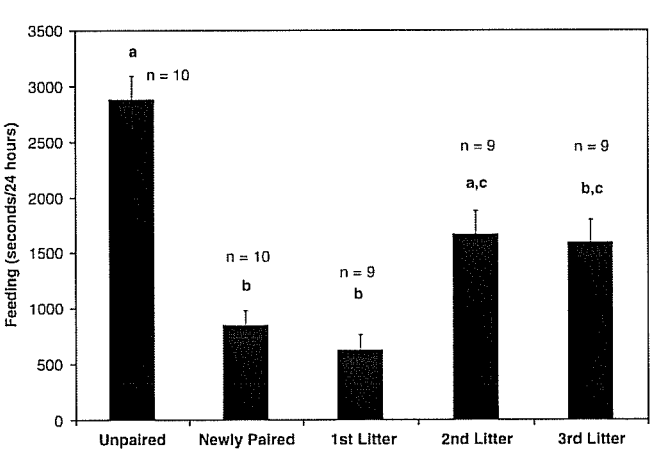

Fig. 3. Effects of social condition on the duration of time (mean ± S.E.; in seconds) spent in feeding behavior during a 24 hour period ($F_4 = 5.79$, $p = 0.001$). Groups which are statistically different according to post hoc testing are marked with different letters; groups marked with the same letters are not statistically different.

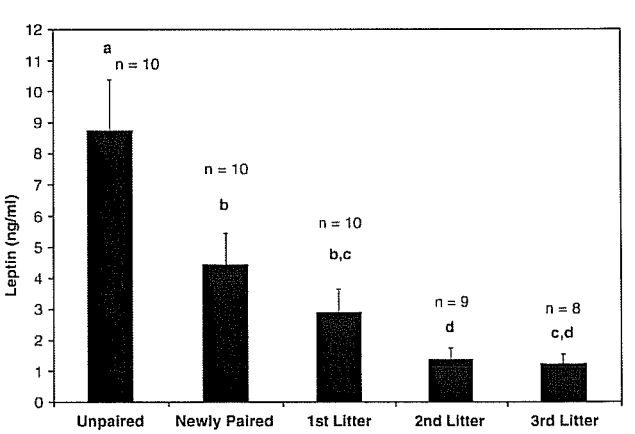

Fig. 4. Effects of social condition on plasma leptin levels (mean ± S.E.). Variation in sample size was due to inability to obtain bleeds from some animals at that time point. Groups which are statistically different according to post hoc testing are marked with different letters; groups marked with the same letters are not statistically different.

approached statistical significance ($F_2 = 3.30$, $p = 0.054$), with lower mean weight in males that were paired for 3 days.

3.2.2. Leptin levels

Consistent with Experiment I, we found in Experiment II that social condition significantly affected males' circulating leptin concentration ($F_2 = 4.75$, $p < 0.019$), with leptin being significantly reduced following pairing and parenting (Fig. 6). Post hoc analysis showed differences between unpaired males and both paired conditions (newly paired males, $t = 2.94$, $p = 0.007$; paired 17 days after the first litter, $t = 2.21$, $p = 0.037$). There was a positive correlation between plasma leptin concentrations and the weight of each of the three fat pad weights (perirenal, $r = 0.65$, $p = 0.0003$; subcutaneous, $r = 0.81$, $p = 0.0001$; epididymal, $r = 0.73$, $p = 0.0001$). Since social condition primarily affected subcutaneous fat pad weight, we conducted separate leptin × subcutaneous fat pad correlation analyses for each social condition. For each of the 3 social conditions, there was a significant positive correlation between plasma leptin and subcutaneous fat pad weight (unpaired, $r = 0.85$, $p = 0.017$; newly paired, $r = 0.92$, $p = 0.0001$; paired 17 days after first litter, $r = 0.89$, $p = 0.001$). However, compared to unpaired males, in paired males at 17 days after first litter, the slope of the regression equation for leptin and subcutaneous fat was significantly enhanced ($F_1 = 16.31$, $p = 0.0006$).

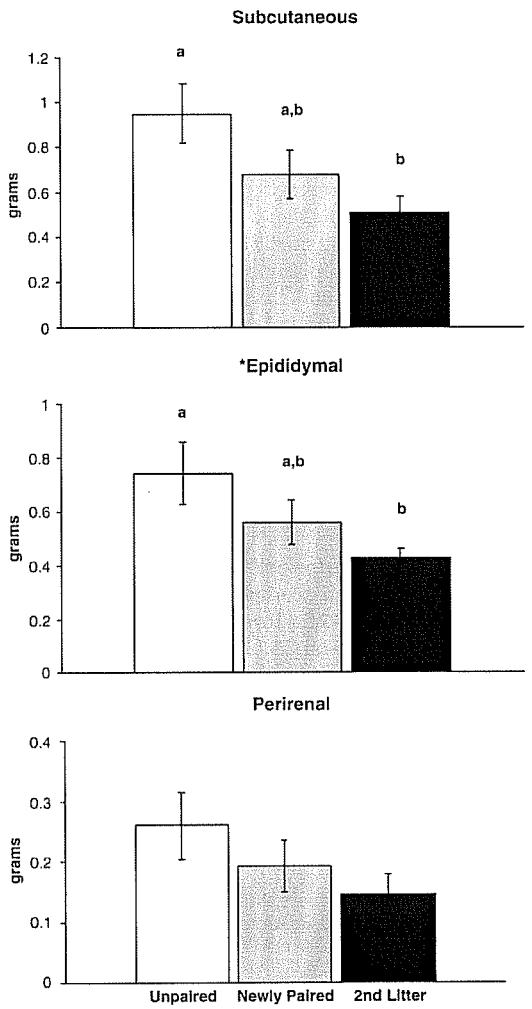

Fig. 5. Effects of social condition on fat pad weights (overall ANOVA subcutaneous: $F_2 = 3.84$, $p = 0.036$; *epididymal tended to be different: $F_2 = 3.02$, $p = 0.0675$). Groups which are statistically different according to post hoc testing are marked with different letters; groups marked with the same letters are not statistically different.

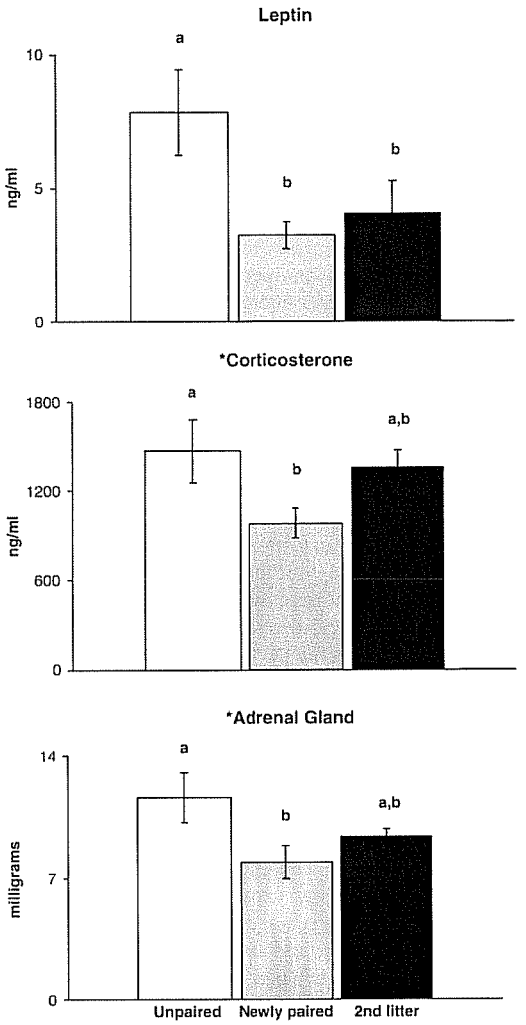

Fig. 6. Effects of social condition on leptin and adrenocortical activity (overall ANOVA leptin: $F_2 = 4.75$, $p < 0.019$; corticosterone: *corticosterone tended to be different: $F_2 = 3.08$, $p = 0.064$; *adrenal weight tended to be different: $F_2 = 3.30$, $p = 0.0542$). Groups which are statistically different according to post hoc testing are marked with different letters; groups marked with the same letters are not statistically different.

3.3. Corticosterone levels

Social condition tended to affect circulating corticosterone concentrations ($F_2 = 3.08$, $p = 0.064$). This tendency to affect corticosterone resulted primarily from a mean corticosterone concentration decrease in newly paired males (Fig. 6), which is consistent with the effect on adrenal weight. There was no correlation between corticosterone levels and leptin levels. Although no significant correlation existed between plasma corticosterone and each of the 3 fat pad weights, when analyzed for each of the social conditions, only males 17 days after first litter showed a significant and positive correlation between plasma corticosterone and subcutaneous fat pad weight (paired 17 days after litter birth, $r = 0.85$, $p = 0.008$).

4. Discussion

In this project, we provided evidence that male prairie voles experience a cost to engaging in social behavior (pairing and parenting), demonstrated by significant losses in body weight and fat reserves. This suggests either an increase in energy expenditure and/or a decrease in energy intake during the mating and parenting

process of the male prairie vole. Our behavioral data also suggested a decrease in energy intake during certain conditions, in particular when males were newly paired and prior to the birth of the first litter. In addition, males tend to spend more time in nest-building in all conditions subsequent to mating, although this was not significant. Early infant care, which was not assessed in this study, may very well account for the additional energy expenditure. One interesting finding regarding social effects on weight loss in our subjects was the fact that their lowest weights on average were recorded just before the birth of the 2nd litter. This suggests that perhaps the paternal investment in the first litter may be a greater energy cost to males than subsequent litters. Feeding behavior at this time point is higher than before the birth of the first litter, which could be compensatory for energy expended while raising that litter.

Perhaps the most important finding of this study is that engaging in social behavior resulted in costs for males even when in a situation of limited space, no predation, and *ad libitum* food. In the wild, energetic costs associated with increased need for foraging, possibly limited food supply, and the need for vigilance could easily make the costs of social behavior much more substantial than in this study, perhaps leading to decreased survivorship for paired males [10]. This may increase the benefits of becoming a "wandering" male, which is a common (45% of adult males) alternative male strategy [31] but which in short-term studies does not seem as successful as being a paired male [32].

In both the longitudinal and cross-sectional studies, we also found significant drops in leptin levels in males after being paired with a female. Leptin, an important metabolic hormone, is secreted by fat cells and is known to regulate food intake and body weight. Higher fat content in the body typically leads to higher levels of plasma leptin, which signal the status of adipose stores to the CNS [33]. Leptin is thought to regulate long-term energy balance, and it acts to attenuate appetite and increase energy expenditure through its effects on neurotransmitters in the arcuate nucleus of the hypothalamus. In addition to effects on hunger and satiety in adult animals, during the neonatal period, leptin is involved in the development of a neural circuitry that involves the arcuate nucleus, paraventricular nucleus, lateral hypothalamic area, and dorsomedial hypothalamic nucleus, regions in the brain that regulate food intake and overall energy balance [34]. Consistent with its role as a signal of energy resources (stores), when insufficient levels are present, it has been observed to serve as a metabolic signal to discontinue estrus and, therefore inhibit a series of behaviors that would be otherwise very energetically costly [35].

Based on the drops in body weight and leptin that we saw in the first experiment, we tested in a subsequent experiment whether lower fat reserves might explain the decreases in body weight and leptin observed in Experiment I. Indeed, we did find that in paired males 17 days after the birth of their first litter, subcutaneous fat pad weights were significantly reduced. Although circulating leptin concentrations positively correlated with fat pad weight under each of the three social conditions, we found that the slope of this relationship between leptin and subcutaneous fat was enhanced in paired males 17 days after litter birth. Moreover, only in these males did we find a significant and positive association between plasma corticosterone and subcutaneous fat pad weight. Together, these results strongly suggest that, for males, investment in social behaviors may be part of an orchestrated shift in energy signaling that leads to reduced drive for and time spent in behaviors like foraging. In turn, there becomes greater dependence on internal energy reserves leading to negative energy balance.

Our sucrose data suggest that in this case, this measure did not reflect affective state or psychological stress [36,37]. The most straightforward explanation of the increased sucrose intake is one of energy economy, whereby an emphasis is placed on the intake of calorically dense foods that provide a quick source of energy to meet metabolic demands during a period when mating and parental care are critical. It is possible that the increase in sucrose preference was related to an increased reward value of the sucrose mediated by removal of the inhibitory effects of leptin on dopaminergic neurons. This fits with the proposed role of leptin as a regulator of food intake though modulation of reward [38–40]. This also suggests that the increase in sucrose preference over the course of social change is a product of the drop of leptin caused by weight loss, and not related to either affective state or to behavioral attempts to regulate the neuroendocrine effects of increased stress. However, given the relatively few times that corticosterone was measured and the limited exposure to the sucrose solution, it is not possible to rule out transient changes in corticosterone as a driver of the sucrose intake.

One caveat for the longitudinal study presented here is that we did not have a control group of males which remained unpaired for the length of the study. Thus, males could have lost weight due to age or repeated handling rather than social condition. However, we think this is unlikely because in the cross-sectional study, handling experience was identical between groups and animals were sacrificed within a very small age window (104–120 days). In addition, body weight loss was not monotonic but had started to recover by the final endpoint of the longitudinal study. However, for future studies addition of this control group would be valuable.

In summary, the data from this experiment support the hypothesis that metabolic needs do in fact change in response to pairing and parenting in male prairie voles, and that males may lose fat stores due to reduced feeding after these social changes. That is, our findings suggest that central orchestration of social behavior in prairie voles is tied to the central regulation of energy balance. If true, shifts in social behavior may in fact depend on and be explained by energetic status (fat storage) and/or energy availability (e.g., access to calorically dense foods). Since these energetic-related variables can shift quite dramatically (in either direction), the implications to behavioral economy and success are potentially large. Overall, our results reveal a new dimension with which to assess behavioral change in the prairie vole and support previous work in other species that demonstrates a link between energy balance and social behavior. Further research should continue to elucidate the hormonal and physiological consequences of changes in social condition in male mammals. Monogamous species make excellent models for human behavior, in which males are often significant contributors to infant care and potentially incur significant costs.

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